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THE UNIVERSITY OF ALBERTA

STUDIES ON DIGESTION OF NUTRIENTS AND RATE OF PASSAGE IN THE HORSE

by

LEONARD KENNETH TODD

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

ANIMAL NUTRITION

DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF ALBERTA

EDMONTON, ALBERTA

SPRING 1986

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THE AULD FARMER'S NEW-YEAR-MORNING SALUTATION TO HIS AULD MARE, MAGGIE

On giving her the accustomed ripp of corn to hansel in the New Year.

by Robert Burns

A GUID New-year I wish thee, Maggie!
Hae, there's a ripp to thy auld baggie:
Tho' thou's howe-backit now, an' knaggie,
I've seen the day
Thou could hae gaen like onie staggie,
Out-owre the lay.
Tho' now thou's dowie, stiff an' crazy,
An' thy auld hide as white's a daisie,
I've seen thee dappl't, sleek an' glaizie,
A bonie gray:
He should been tight that daur't to raize thee,
Ance in a day.
Thou ance was i' the foremost rank,
A filly buirdly, steeve an' swank;
An' set weel down a shapely shank
As e'er tread yird;
An' could hae flown out-owre a stank
Like onie bird.
It's now some nine-an'twenty year
Sin' thou was my guid-father's meere;
He gied me thee, o'tocher clear,
An' fifty mark;
Tho' it was sma', 'twas weel-won gear,
An' thou was stark.
When first I gaed to woo my Jenny,
Ye then was trotting wi' your minnie:
Tho' ye was trickie, slee, an' funnie,
Ye ne'er was donsie;
But hamely, tawie, quiet, an' cannie,
An, unco sonsie.
That day, ye pranc'd wi'muckle pride,
When ye bure hame my bonie bride:
An' sweet an' gracefu' she did ride,
Wi' maiden air!
Kyle-Stewart I could bragged wide
For sic a pair.
Tho' now ye dow but hoyte and hobble,
An' wintle like a saumont coble,
That day, ye was a jinker noble,
For heels an' win'!
An' ran them till they a' did wauble,
Far, far, behin'!
When thou an' I were young and skeigh,
An' stable-meals at fairs were dreigh,
How thou wad prance, and snore, an' skreigh
An' tak the road!
Town's-bodies ran, an' stood abeigh,
An' ca't thee mad.
When thou was corn't an' I was mellow,
We took the road ay like a swallow:
At brooses thou had ne'er a fellow,
For pith an' speed;
But ev'ry tail thou pay't them hollow,
Whare'er thou gaed.

The sma', droop-rumpl't hunter cattle
Might aiblins waur't thee for a brattle;
But sax Scotch mile, thou try't their mettle,
An' gar't them whaizle:
Nae whip nor spur, but just a wattle
O' saugh or hazle.
Thou was a noble fittie-lan',
As e'er in tug or tow was drawn:
Aft thee an' I, in aught hours' gaun,
On guid March-weather,
Hae turn'd sax rood beside our han'
For days thegither.
Thou never braing't, an' fetch't, an' fliskit;
But thy auld tail thou wad hae whiskit,
An' spread abreed thy weel-fill'd brisket,
Wi' pith an' power;
Till sprittie knowes wad rair't an' riskit
An' slypet owre,
When frosts lay lang, an' snaws were deep
An, threaten'd labour back to keep,
I gied thy cog a wee bit heap
Aboon the timmer:
I ken'd my Maggie was na sleep
For that, or simmer.
In cart or car thou never reestit;
The steyest brae thou was hae fac't it;
Thou never lap, an' sten't, and breastit,
Then stood to blaw;
But just thy step a wee thing hastit,
Thou snoov't awa.
My pleugh is now thy bairn time a',
Four gallant brutes as e'er did draw;
Forbye sax mae I've sell't awa,
That thou hast nurst:
They drew me thretteen pund an' twa,
The vera warst.
Monie a sair darg we twa hae wrought,
An' wi' the weary warl' fought!
An' monie an anxious day I thought
We wad be beat!
Yet here to crazy age we're brought,
Wi' something yet.
An' think na', my auld trusty servan',
That now perhaps thou's less deservin,
An' thy auld days may end in starvin;
For my last fow,
A heapit stimpart, I'll reserve ane
Laid by for you.
We,ve worn to crazy years thegither;
We'll toyte about wi' ane anither;
Wi' tentie care I'll flit thy tether
To some hain'd rig,
Whare ye may nobly rax your leather,
Wi' sma' fatigue.

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled STUDIES ON DIGESTION OF NUTRIENTS AND RATE OF PASSAGE IN THE HORSE submitted by LEONARD KENNETH TODD in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL NUTRITION.

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Supervisor

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Date April 25 '86

General Abstract

Several studies on digestion of nutrients and rate of passage were conducted with horses at the University of Alberta Large Animal Metabolic Unit.

In the first experiment, eight mature geldings with average weight of 523 kg (standard deviation of ±39.5) were fed four different physical forms of alfalfa (long, chopped, cubes and pellets) that was harvested from the same field and at the same stage of growth. The nutrient digestibilities and the voluntary intake were determined according to a replicated Latin Square design. The alfalfa was supplied in two equal portions of 3.7 kg, at 0800 and 2000 h, according to the daily digestible energy(D.E.) maintenance requirement for a non-working horse which is defined by N.R.C. (1978) as $D.E.(MJ/day)=0.649W^{0.75}$. Dysprosium (Dy) was used as the digestibility marker and supplied as part of vitamin-mineral pellets, which were fed to the horses in 250 g portions prior to feeding. There were no differences ($p>0.05$) between the digestibilities of dry matter, energy, crude protein and neutral-detergent fiber among the different forms of alfalfa, irrespective if these were based on total collection or on Dy. The digestibilities of nutrients based on Dy were lower ($p<0.05$) than those based on total collection. Voluntary intake trials were carried out and only the last 4 days of the 6 day voluntary intake trial were considered. The voluntary intake of cubes were highest ($p<0.05$) and there were no differences ($p>0.05$) in the voluntary intake between the other forms of alfalfa.

In the second experiment, six mature geldings with average weight of 521 kg (standard deviation of ±47.5) were used to determine the effect of level of feed intake on the digestibilities of dry matter, energy and protein. The experiment was carried out according to a randomized block design. Each experimental period included 14 days of adaptation prior to a 6 day collection period of feces. Alfalfa cubes were supplied according to the maintenance requirement for digestible energy which was calculated for each horse individualy as $D.E.(MJ/day)=0.649W^{0.75}$ and also according to 80% of their *ad libitum* feed intake as measured in experiment one. The cubes were supplied in two equal portions, at 0800 and 2000 h. Chromic oxide (Cr_2O_3) was used as digestibility marker and supplied as part of the

vitamin-mineral pellets, which were fed in 250 g portions prior to feeding. There were no differences ($p>0.05$) in the digestibilities of dry matter, energy and protein at the two levels of feed intake, irrespective if these were determined by aid of the total collection or Cr_2O_3. The digestibilities of nutrients based on Cr_2O_3 were similar to those based on total collection. In addition, studies were carried out to determine the effect of level of feed intake on rate of passage. There were differences ($p<0.05$) in mean retention times, which were 15.6 and 25.2 h, when the cubes were supplied at the high and low level of feed intake, respectively.

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I. General Introduction

Nutrition in the horse presents different problems from that of the ruminent. The latter performs most of the fiber fermentation in a large rument which is part of the stomach. Horses, however have a small, simple stomach and fermentation, along with absorption of some of the nutrients (such as energy and vitamins) takes place in the large colon and cecum in the hindgut. Thus there is considerable interest in studying digestion in these animals.

Alfalfa is increasingly being used as a feedstuff for horses. The prejudice that used to exist against alfalfa has disappeared as research evidence and experience with alfalfa hay indicates it is an excellent feedstuff for horses (Cunha, 1980; Wagoner, 1973). Nutrient digestibilities in alfalfa hay have been reported by several research groups (Fonnesbeck, 1967; Van der Noot and Gilbreath, 1970 and Haenlein *et al.*, 1966). However, there is a scarcity of information on the nutritive value of different physical forms of alfalfa, such as pellets and cubes. These forms of alfalfa, because of the many conveniences associated with its use, are used more and more in rations for horses. In the first experiment, the digestibilities of nutrients, in addition to the voluntary intake, were measured in different forms of alfalfa, namely long, chopped, cubes and pellets.

There is little information on the effect of the level of feed intake on nutrient digestibilities in horses since nutrient digestibilities are usually determined at maintenance levels of feeding. It is important to have information on the effect of level of feed intake on nutrient digestibilities and rate of passage. This was investigated in horses fed alfalfa cubes in the second experiment.

Digestibility studies with horses, based on total collection, require a large amount of labour and equipment (Van der Noot *et al.*, 1970). Furthermore, the horses can be kept in a normal state of activity rather than being confined to a metabolic crate when digestibility markers are used for the determination of nutrient digestibilities (Parkins *et al.*, 1982). As part of the two experiments, studies were carried out to determine the validity of using dysprosium, a rare-earth element, and chromic oxide as digestibility markers.

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II. THE DIGESTIBILITIES OF FOUR DIFFERENT PHYSICAL FORMS OF ALFALFA HAY AND VOLUNTARY INTAKE IN HORSES

A. Introduction

Alfalfa is increasingly being used as a feedstuff for horses. The prejudice that used to exist against alfalfa has nearly disappeared but a few examples remain. For instances, one example of prejudice is personal communication that was experienced with a veternarian from Red Deer, Alberta, who stated that if he wanted to slow down a race horse all he had to do was feed it a "flake" from a bail of alfalfa hay. In another example, the author Wagoner (1973), who highly favours alfalfa as a feed for horses, has repeated a statement by some alfalfa growers who say that if alfalfa is cut before full bloom, the hay will be highly laxative to horses. Research evidence and experience with alfalfa use has indicated that it is an excellent feedstuff for horses (Cunha, 1980).

Nutrient digestibilities for alfalfa hay have been reported by Fonnesbeck *et al.* (1967), Haenlein *et al.* (1966a,b) and Van der Noot and Gilbreath (1970). However, with the exception of studies by Haenlein *et al.* (1966a,b), there is a scarcity of information on the nutritive value of different physical forms of alfalfa, such as cubes and pellets. These forms of alfalfa, for reasons of the many conveniences associated with its use, are used more and more in rations for horses. Furthermore, the results that were obtained by Haenlein *et al.* (1966a,b) were inconclusive. Based on total collection, the digestibilities of nutrients were higher in long hay than in pellets and wafers(cubes). However, based on a digestibility marker (chromic oxide) the digestibilities were higher in pellets and wafers than in long hay.

In addition to determining the nutritive value of the different physical forms of alfalfa, the present studies were carried out to determine the feasibility of Dysprosium(Dy), a rare-earth element, as a digestibility marker. Digestibility studies with horses, based on the total collection method, require a large amount of labour and equipment. Furthermore, the horses can be kept in a normal state of activity rather than being confined to a metabolic crate when digestibility markers are used for the determination of nutrient digestibilities.

Chromic oxide has been used traditionally as a digestibility marker in most domestic animal species. The performance of chromic oxide as a marker in horses was found to be less than ideal even though recovery was nearly complete, in the studies of Haenlein *et al.* (1966a,b) and less adequate than other markers in the study by Knapka *et al.* (1967). The use of rare-earth elements in digestibility studies offer promise because of their property of strong binding to forages particles. Knapka *et al.* (1967), in studies with burros, showed that ^{144}Ce gave nutrient digestibility measurements that were similar to those obtained by total collection. Recently, Kennelly *et al.* (1980a,b) showed the feasibility of using Dy (in the form of $DyCl_3 \cdot 6H_2O$) as a digestibility marker in swine. In addition, Dy concentration can be rapidly determined by neutron activation analysis which provides a sensitive method for analysis.

The objectives of the present studies were to determine the voluntary intake and the nutrient (dry matter, crude protein, energy and neutral-detergent fiber) digestibilities in different physical forms of alfalfa (long, chopped, pellets and cubes) and the validity of using Dy as a digestibility marker in studies with mature geldings.

B. Materials and Methods

Eight mature geldings with average weight of 523 kg (standard deviation of ± 32.5) were fed alfalfa that was harvested from the same field and at the same stage of growth. The four different physical forms of alfalfa: long, chopped, cubes and pellets were measured for nutrient digestibilities according to a replicated Latin square design. Each experimental period included 14 days of adaptation to each particular form of alfalfa. The horses were given 7.4 kg of alfalfa and 500 g of a vitamin-mineral pellet mixture each day (table II.1). The alfalfa was supplied at the level of the digestible energy(D.E.) requirement for maintenance for a 500 kg mature non-working horse according to N.R.C.(1978) as defined as

$D.E.(MJ/day) = 0.649W^{0.75}$.

The pellets(table II.1) supplied most of the common vitamins and minerals recommended by N.R.C.(1978)(table II.1). A rare-earth digestibility marker, Dy, was added to the vitamin-mineral pellets to give a final dietary concentration of 25 ppm. Dry molasses

was included in the vitamin-mineral pellets to make this portion of the ration more appealing to the horse. The vitamin-mineral pellets were fed prior to the alfalfa ration to ensure that all of the Dy that was supplied at each feeding was completely consumed. The horses were fed twice daily, at 0800 and 2000 h. The horses were kept in partly covered individual pens outdoors during the 14 day adaptation period. The horses were then moved indoors into metabolic cages for 6 days for a total collection of feces and urine. Urine was collected separately from feces by the aid of a funnel-harness apparatus which was attached to the horse and which drained the urine into collection pails (plate II.1). Feces were collected by placing a large plastic tub at the rear of the cage. The metabolic cages were constructed of steel and the floor was constructed from heavy wooden planks on which the horses were held 20 cm above the floor of the barn. A removable wooden feeder was placed inside the cage at the front which had a volume capacity for hay on the inside bottom and an upper shelf(plate II.2) for holding the vitamin-mineral pellets and a pail for water which was filled up at least three or four times daily. Trace-mineralized salt blocks were supplied on the bottom of the feeder. Approximately 40 g of boric acid powder was added to the urine collection pails prior to collection in order to inhibit bacterial growth and prevent the loss of nitrogen. During the six day collection period, the feces were weighed at 0800 h each day, mixed thoroughly, and a subsample of approximately 500 g was collected from random spots in the tub. The urine was mixed, weighed and a subsample was collected as well. Samples were immediately placed in a freezer at a temperature of -20°C. After the six day collection period, the horses were moved to the outside pens for the measurement of the voluntary intake of each physical form of alfalfa. During this time the horses remained on the same ration except that the feed was supplied four times daily to ensure that it was always available. Vitamin-mineral pellets were not supplied at this time. The feeding times for the voluntary intake trial were 0800, 1200, 1600, and 2000 h. The voluntary intake trial was carried on for 6 days but only the data from the last 4 days were used because the intake level was relatively steady here compared to the first two days at the start of each intake trial. Only three voluntary intake trials are reported in this paper because insufficient hay was available to complete the last digestibility trial. The

horses were moved to an open pasture for 2 days after the voluntary intake trials and then moved back to the outside pens to start the next experimental period. During the time the horses were kept at the University of Alberta farm, they were administered routine vaccines and given anthelmintics if parasite eggs were found in excess numbers in the feces. In addition, the horse's feet were checked, and treated if necessary, at regular intervals.

The average size of the different physical forms of alfalfa feed were as follows: cubes, 3.3 cm x 3.3 cm x 3.3 cm; pellets, 0.7x1.2 cm; chopped hay and and long hay. The alfalfa pellets were prepared commercially by cutting the alfalfa green, followed by flash-drying the material at 200°C, grinding and pressing the material through a die. The cubes were prepared by cutting the alfalfa green, partially wilting the cut plant, drying the material at 200 °C and pressing the material through a die. The vitamin-mineral pellets were prepared from a base of ground oats and 10% dry molasses. The $DyCl_3 \cdot 6H_2O$ hydrated salt was ground to a fine powder and sieved through a screen with a mesh size of 0.5 mm before pre-mixing with approximately 2 kg of vitamins and minerals. The vitamins, minerals, and $DyCl_3$ mixture were then added to a portion (approx. 10 kg) of the total ground oats and molasses to be used. This portion was then mixed with the remaining ground oats and molasses to give the final mixture and pelleted.

Samples of feces were weighed before and after freeze-drying and then ground through a 1-mm mesh screen using a Wiley mill. Samples of feces from each of the 6 days of the collection period from each individual horse were pooled on the basis of daily dry matter excretion. The pooled samples were then analyzed for dry matter, crude protein and energy according to A.O.A.C. (1980). Neutral-detergent fiber was analyzed according to the method of Goering and van Soest (1970). The four forms of alfalfa and vitamin-mineral pellets were also analyzed for dry matter, crude protein, energy and neutral-detergent fiber.

Instrument neutron activation analysis (I.N.A.A.) using the SLOWPOKE II nuclear reactor at the University of Alberta was selected as the method to determine the Dy concentration in vitamin-mineral pellets and feces because of the advantages and conveniences described by Kennelly *et al.* (1980 a,b). One of the advantages of using neutron activation for

measuring Dy is the small amount that needs to be added to the diet. In order to choose a marker that can be quantified at low levels, the element should have a high nuclear cross-sectional or activation reactivity. ^{164}Dy has one of the highest nuclear cross-sections among the lanthanide isotopes. Most of the problems of interference in Dy detection are caused by activated radionuclides of Mn, Cl, and Na that occur naturally in biological materials. A 45 sec-30 sec-60 sec irradiation, decay, counting scheme was chosen by Kennelly *et al.* (1980b) to selectively determine the content of the gamma ray producing nuclide-^{165}Dy^{m}(half-life=75 sec). This scheme reduces the interference from other elements in the sample because of the short irradiation time. If another nuclide such as ^{165}Dy (half-life=2.3 hr) had been chosen for detection then, because of the longer irradiation time required, it would be necessary to employ a longer cooling time to permit the decay of other elements which would cause interference (Kennelly *et al.*, 1980b).

To prepare the sample for irradiation and counting, 700 to 900 g of pooled freeze-dried samples of feces were weighed into 7 mL polyethelene vials. Approximately 20 mg of ground vitamin-mineral pellets with ground oats were weighed into vials for determining the Dy content of the vitamin-mineral pellets. The addition of oats was to give the pellet sample a consistant and similar geometry to the fecal samples. The remaining space in the vials were filled with melted paraffin wax. The wax held the sample in a fixed position which helped ensure a constant geometry during the counting time after neutron activation. The vials were hermetically sealed and a plastic cap was pressed over the sealed end as an added safety feature to ensure that there was no loss of sample in the reactor site.

The photopeak used for the analysis of Dy was from the 108 keV gamma ray emission produced from the decay of ^{165}Dy^{m}. The raw gamma ray count was automatically corrected for "dead time" errors that occur when gamma ray activities are strong which causes the spectrometry system to overload and not process all gamma rays from the sample. The time lost in counting is called "dead time" and the equivalent is added on to the pre-set counting time(60 seconds for measuring ^{165}Dy^{m}). The problem becomes compounded when, during counting, the radioisotope of interest is reduced in quantity due to decay. In this case, the

additional counting time would not adequately compensate for loss of counts because the remaining quantity of isotope would not give an appropriate number of counts. A correction factor equation was applied to mathematically add the appropriate counts that would otherwise be lost when the isotope decays at the end of the preset "clock time":II.1:

$$\text{Equation II.1} \quad C = C' \times \frac{CT}{LT} \left[\frac{1-e^{-0.693CT/t^1/2}}{1-e^{-0.693LT/t^1/2}} \right]$$

This equation was taken from the work of Takeuchi *et al.* (1980): where C is the corrected counts, C' is the raw counts, LT is the live time, CT is the clock time (CT=LT+DT), DT is dead time and $t^1/2$ is the half-life of $^{165}Dy^m$. The correction factor is useful for samples with different levels of Dy which would otherwise be measured with varying accuracy by the counting instrument that adds "dead time" to the pre-set "count time". The end result is a consistent measurement of counts and therefore a consistent measurement of Dy.

If the "dead time" is greater than 10% of "clock time" then a second potential source of error, namely "pulse pile-up" occurs. This happens when two signals are detected almost simultaneously and processed as a new signal. The "pulse pile-up"error becomes significant if the "dead-times" are greater than 10% of the "clock times". In the present studies all "dead times" were below 10%. Therefore it was not necessary to apply a correction for this factor.

The corrected gamma ray counts per weight of sample were used directly to calculate the digestibilities of nutrients. The contents of Dy are directly proportional to the gamma ray count of the photopeak 108 kev according to Kennelly *et al.* (1980a,b) and because of the standard of quality control of measurement at the SLOWPOKE facility. To calculate digestibility of nutrients(or apparent digestibility), the quantity of nutrient ingested that was not recovered in the feces, was expressed as a percentage of the intake of nutrients.

When calculating digestibilities using the total collection method, the daily intake of the diet has to be constant and the numbers of collection days sufficiantly long so that the output of feces also will remain constant. This will ensure that the feces collected between fixed time intervals(i.e. from 0800 h from one day to the next) will contain the output from

an intake over an equal time period. The collection period in this study lasted six days so that the day-to-day fluctuations would balance out.

When using a digestibility marker or a "index substance" for the measurement, the digestibility of nutrients in the diet are determined from the ratio on the concentration of the index material in the feed to the concentration in the corresponding fecal output. Also included in the digestibility calculation is the concentration of the nutrient(to be measured) in the feed and the feces. The Dy content (or the gamma ray counts) of the vitamin-mineral pellets was used for the calculation of the Dy content of the total diet(vitamin-mineral pellets plus alfalfa). The digestibilities of dry matter, gross energy, crude protein and neutral-detergent fiber were determined by means of both the total collection and the marker. Digestibility coefficients using Dy as a marker were calculated according to the following equation:II.2:

$$\text{equation II.2} \quad \text{Apparent Digestibility} = 100 - \left[100 \times \frac{\%\text{Marker in Feed}}{\%\text{Marker in Feces}} \times \frac{\%\text{Nutrients in Feces}}{\%\text{Nutrients in Feed}}\right]$$

The data for each response criterion were subjected to Least Squares analyses of variance (Steel and Torrie, 1980).

C. Results

The four physical forms of alfalfa were very similar in chemical composition. There were no differences in the content of dry matter, crude protein, gross energy, ash, crude fiber and neutral-detergent fiber among the different physical forms of alfalfa (table II.2). The chemical composition of the vitamin-mineral pellets is shown in table II.3.

The horses readily consumed the various forms of alfalfa and there were no noticeable health or feeding problems during the experiment with the exception of horse number 8 showed mild signs of "colic" shortly after consuming part of the alfalfa pellets. This horse recovered later when he was given a simple remedy treatment (the horse was given a common liquid stomach medicine, Pepto-Bismol®, and walked for half a hour). The horses had no difficulty chewing the cubes or pellets. The horses maintained their weight within 6% of their

initial weight throughout the experiment (table II.4). Water consumption was not effected by diet although it differed with each horse. Two of the horses (numbers 3 and 4) consumed a greater amount of water compared to the other ones; accordingly, urine output was increased in these horses.

Most horses stabilized their intake after the first 2 days during the voluntary intake trials. The horses consistently consumed more during the first day as compared to the remaining five days. The last 4 days were considered in the determination of the voluntary intake. The voluntary intake of the cubes was higher than the intakes of the other forms of alfalfa ($p<0.05$),(table II.5). There were no significant differences in voluntary intake between long, chopped and pelleted hay ($p>0.05$). There were significant differences among individual horses. Horse number 5 consistently consumed a greater amount of all diets. This was reflected by a significant animal effect in the analysis of variance for voluntary intake (table II.7).

The digestibilities of dry matter, crude protein, gross energy, and neutral-detergent fiber were similar($p>0.05$) among the different physical forms of alfalfa when measured by the total collection method (table II.5). The digestibility coefficients of nutrients measured with Dy were consistently lower than based on the total collection method (tables II.5 and II.6). There were no significant differences in the digestibilities of nutrients ($p>0.05$) between periods when measured by total collection (tables II.8 and II.9) but there were differences between periods when measured by Dy (tables II.9 and II.10).

Urine from horse number 1, which was fed alfalfa hay and Dy-containing vitamin-mineral pellets during the first total collection period, was freeze-dryed and analysed for Dy by the I.N.A.A. method. There was no detectable Dy found in samples of weight 50 mg. The limits of detection of neutron activationed elements can be calculated by the formula: $L_D = 2.71 + 4.65(C_B)^{0.5}$ (Currie, 1968), where C_B is the background counts. The limit of detection for the 50 mg freeze-dryed urine samples was calculated to be 1.27 ppm. The dry matter content of the urine from horse number 1 was 7.9%. The daily urine output was 12.8 kg so then the dry urine output was 1010 g/day. If 1.79 ppm is the detectable limit

then the maximum amount of Dy that could be lost by way of urine excretion without being detected would be: 1010g/day X 1.79 ppm = 1.8 mg/day. This potential loss from urine is very small in comparision to the daily intake of Dy which was 198 mg.

D. Discussion

There were no significant differences between the digestibility coefficients of dry matter, crude protein, gross energy and neutral-detergent fiber($p>0.05$) among the different forms of alfalfa (tables II.5 and II.6). These results reflect the chemical composition of the different forms of alfalfa which show no differences in dry matter, crude protein, gross energy, acid-detergent and neutral-detergent fiber content.

Results from the literature on the digestibility of nutrients in alfalfa have been compiled in table II.11. Differences in the digestibilities of nutrients can be attributed mostly to the quality of the hay which is dependent in part on the stage of growth at the time of harvesting. The digestibilities of nutrients in alfalfa harvested at the pre-bloom stage of growth are higher than those at bloom stage which is due to the greater leaf content and the lower content of fiber in the younger plant. Other effects such as soil conditions, rainfall and the purity of the crop will also effect the digestibility of nutrients in alfalfa hay. The dry matter and crude protein digestibilities in alfalfa hay of good quality for mature horses would be about 60 and 75%, respectively (table II.11). The digestibilities of dry matter and crude protein in hay of lower quality would be about 50 and 65%, respectively.

The results of the present studies differ from those of Haenlein *et al.* (1966b) who observed, that the digestibilities of nutrients were higher in pellets and wafers(cubes) than in long alfalfa hay based on chromic oxide. In his studies, however, the total collection digestibilities of nutrients were higher in long alfalfa hay than in pellets and wafers. Haenlein *et al.* (1966b) also found that the digestibilities of nutrients in all forms of alfalfa were slightly lower when these were based on chromic oxide than on total collection. The differences in nutrient digestibilities, based on chromic oxide, between pellets, wafers and long hay may be related to differences in the kinetics of marker excretion which occur when there

are differences in voluntary intake among the different forms of alfalfa (intake levels were not reported).

The results of the present study gave lower nutrient digestibilities based on Dy than on total collection. However, there were no differences in nutrient digestibilities among the different physical forms of alfalfa when either the total collection or the Dy marker method was used (tables II.5 and II.6). In the present studies the horses were fed 7.4 kg of feed per day according to the recommended maintenance requirement for digestible energy (N.R.C., 1978). Furthermore, the studies by Haenlein *et al.* (1966b) showed a higher voluntary intake of pellets and wafers than of long hay. In the present studies, the voluntary intake of only the cubes was higher than of long hay. One reason for the differences in digestibilities and voluntary intake between the studies may be due to the fact that Haenlein *et al.* (1966b) used ponies. Mature horses were used in the present studies. Another reason for the differences in nutrient digestibilities reported by Haenlein *et al.* (1966b), based on chromic oxide, may be caused by the effect of the *ad libitum* intake. At the *ad libitum* level of feed intake, mixing of the marker within the cecum and the large intestine may not be as complete as when the feed intake is lower especially when the marker is administered in a concentrated form such as in a capsule. Retrograde mixing in the hindgut may be more complete at restricted levels of feed intake because there is less volume of digesta to be moved. *Ad libitum* feeding in the studies by Haenlein *et al.* (1966b) produced an apparent diurnal fecal marker concentration excretion pattern that was related to the length of time between morning and evening feedings. The degree of mixing of the marker and digesta due to the differences in *ad libitum* intake of pellets, wafers and long hay may have caused the differences in nutrient digestibilities when these were based on chromic oxide. A more constant excretion of chromic oxide was found in studies by Parkins *et al.* (1982) in which horses were fed at maintenance levels. Chamberlain and Thomas (1983), in studies with cows, found an improvment in oxide recovery and a more constant excretion if the marker was mixed with a portion of the diet rather than administered as chromic oxide paper given twice daily. Chromic oxide paper was found to be satisfactory with sheep in their studies.

The use of Dy as a digestibility marker in horses was found to be unsatisfactory in the present studies. The marker was administered in two equal parts in the form of vitamin-mineral pellets. The nutrient digestibilities based on Dy were lower than those based on total collection, which is in agreement with studies by Young *et al.*(1976) in studies with steers. However, Ellis (1968) found similar nutrient digestibilities based on Dy and total collection in sheep. The results of the studies by Ellis (1968) are doubtful because he only used three sheep and then disqualified one. Knapka et al. (1967), in studies with burros, found lower nutrient digestibilities based on chromic oxide as compared to ^{144}Ce and polyethylene particles which gave similar digestibilities to those based on total collection. The recovery of chromic oxide was lower in studies by Knapka *et al.* (1967) than by Haenlein *et al.* (1966b), who used a larger dose of the marker. As was speculated by Knapka *et al.* (1967), the use of a higher dose should reduce the retention, as expressed as a percentage of the administered dose, of the marker. As opposed to Dy in the present study, the recovery of the radioactive rare-earth marker, ^{144}Ce was good (96.5%) when it was administered in a gelatin capsule prior to each feeding.

When given in a concentrated dose (as in the present studies) the mixing of $DyCl_3$ with digesta will depend on the feed intake, the flow rate of the digesta, the volume of the digesta in relation to the volume of the section of the gut in which it is present and the motility of that section of the gut. Little digestion of the vitamin-mineral pellets and the mixing of the digesta with Dy can be expected from the mouth to the proximal duodenum because of the short transit time and the small volume of the stomach. The mixing of saliva with the pellets, because of the high pH, would cause the formation of some Dy-hydroxide (Ellis, 1968). $DyCl_3$ in the stomach would remain as a chloride and some of the hydroxides that were formed would convert back to chlorides because the pH of the contents of the stomach is about 4.7 (Wolter *et al.*, 1980). The pH in the small intestine would range from 6 to 7 (Wolter *et al.*, 1980), therefore Dy would be partly in the form of hydroxides and partly in the form of chlorides. Most of the Dy would pass through the small intestine as a fine precipitate in the form of hydroxides and chlorides that would either remain in solution or

bound to the feedstuff. Most of the precipitates, if these were formed, might move along with the less viscous phase of the mucin layer of the small intestine. The digesta should mix well with the marker in the cecum because of the retrograde movement of the digesta within the cecum. Some of the Dy would probably be converted to hydroxides in this section of the gut because the pH in this region is about 7.3 (Tisserand et al.,1980 ; Ellis, 1968). Any chloride salt that remains will bind tenuously to the fiber (Harber et al., 1981). Hydroxides will accumulate and remain in the physical recesses in the hay particles (Hartnell and Satter, 1979). Further mixing and fermentation on a greater scale would take place in the ventral and dorsal colon. There is a lot of retrograde movement of digesta in this region which preferentially selects the larger particles of hay to keep the colon filled for 48 to 72 hr so that cellulose hydrolysis can occur (Sellers *et al.*, 1979; Sellers *et al.*, 1982). The pH in the colon is approximately 6.2 (Wolter *et al.*, 1980). The phosphate secretions from the colon wall would keep some of the Dy in a precipitated form. The Dy marker then would probably behave similar to chromic oxide so that it would partly adhere to fiber particles and partly be present in the liquid phase of the digesta. Most of the $DyCl_3$ that remains would attach to the finer hay particles and bacteria because of the greater surface area of these substances (Hartnell and Satter, 1979; Combs *et al.*, 1984). Hartnell and Satter (1979) found that $DyCl_3$ binds more readily to fiber than to the starch component of a diet. Insoluble hydroxides were found to be trapped in the hay particles and thought to be adsorbed onto bacteria which were then trapped by hay particles (Hartnell and Satter, 1979). It was shown by qualitative X-ray dispersion studies that the chloride salts of rare-earths elements (Dy, La, and Sa) tended to be more concentrated on the internal rather than on the external surfaces of cell walls of wheat stems (Harber *et al.*, 1981). The low binding capacity of the chlorides on the outer surfaces is due to the silica containing anticlinal cells on the cuticular surface under the conditions of a low pH. The lanthanide element chorides will penetrate the stem and distribute evenly throughout the various tissues such as the ground parenchyma, tracheal tubes and sclerenchyma. Ellis *et al.*(1979) suggested the binding capacities of forages for rare-earth elements to be in the range of 15-25 mg/gm. while the binding capacity for grain was said to

be from 1-4 mg/gm. In addition, some of the transfer of Dy from the vitamin-mineral pellets to the alfalfa hay would be favoured in the stomach because of the low pH.

There may be several reasons for the loss of Dy within the animal including the absorption of the marker into the tissues and fluids of the animal and the accumulation of the marker in the gastrointestinal tract. Miller *et al.* (1967) suggested that some cerium could be absorbed in the digestive tract. Miller and Bryne (1970) found traces of ^{144}Ce in the liver of calves. The accumulation of a marker such as Dy in the form of a precipitate or bound to small particles could occur in the epithelium of the small intestine by the action of "kneading". This action is caused by peristaltic movements of the small intestine and retrograde and mixing motor movements of the ventral and dorsal colon. The "kneading" action was decribed by Volkheimer (1968) as being responsible for the accumulation of starch granules in blood from the gut lumen. He demonstrated in studies with rats that the starch particles moved between the epithelial cells of the small intestine and entered the blood vessels of the deeper mucosal layers in the gut. Human subjects were found to have starch granules in their blood in the range of approximately 1-2 microns after a meal of 200 gm of starch suspended in 800 ml of milk or water (Volkheimer, 1968). Gardner (1984) cited in a review that many types of inert solid particles of several microns in diameter can cross a healthy intestinal wall. The substances that were studied included latex spheres, iron particles, carbon fragments from Indian ink and viruses (Volkheimer,1969; LeFevre and Joel, 1977; Sanders and Ashworth,1961). Chamberlain and Thomas (1983) concluded that chromic oxide accumulates in the stomach of a cow in quantities large enough to cause an incomplete recovery in feces. When Chamberlain and Thomas (1983) administered chromic oxide through a duodenal cannula of three Ayrshire cows they found an improvement in recovery, from 85% to 98%. Other substances that are used as markers, such as silica which is naturally found in feedstuffs, were also found to be retained, especially in ruminants. Van Dyne and Lofgreen (1964) reported fluctuations in fecal silica output in feces in studies with cattle. They considered the use of silica unsuitable as a digestibility marker with cattle. Furthermore, Sutton *et al.* (1977) found a significant variation in the excretion of naturally found silica

(acid insoluble ash) between experimental periods in digestibility trial in horses. If Dy reacts like or is associated with silicious material in the feed then the performance of Dy as a digestibility marker will be similar to that of acid insoluble ash in horses and cattle.

The horses were kept on pasture before the start of the experiment. Thereafter, the horses were fed at a maintenance level for 14 days which may have allowed for the accumulation of silicious material and (or) Dy in the gut wall spaces in the epithelium which can hold particles that are 1-2 microns in size. During the collection period of 6 days, the condition of the gut wall could have prevented further accumulation of Dy as the particle holding capacity of the epithelium in the small intestine and the large colon was saturated and maintained by the restricted level of feeding. The amount of Dy that was excreted during the first collection period was probably sufficient to give digestibility coefficients that were the highest of all four periods and approached values measured by the total collection method (figure II.1). Following the 6 day total collection period of feces, the *ad libitum* feeding trial of 6 days was carried out. The average feed intake during this part of the study was 80% or higher than during the collection period. The higher level of feed intake may have caused conditions in the intestine which could "flush out" any Dy that had accumulated in the lining of the intestine. This tissue, thereafter, would once more be able to accept Dy. In the following digestibility trial (period two), digestibilities based on Dy were significantly lower than based on total collection. Differences in the nutrient digestibilities based on total collection and Dy became even larger during the next 2 periods (tables II.5 and II.6). The causes of Dy retention in the gastro-intestinal tract could be the result of the feeding schedule in which a low level was followed by a high level of feed intake, thereby effecting the condition of the gut so as to vary the degree of retention of Dy. This could occur when Dy is retained in the deep layers of the mucin lining and (or) mucosa during the period of low levels of feed intake during which time Dy would accumulate. Thereafter, Dy would be released during the high level of feed intake. There might have been a progressive increase in the "flushing out" after each *ad libitum* feeding period and which would explain the increasing difference between nutrient digestibilities based on total collection and Dy with each additional

collection period (figure II.1). The mechanisms responsible for accumulation of the marker could be similar to that which happens in ruminants as was observed by Chamberlain and Thomas (1983). As occurred with chromic oxide retention in the rumen, Dy could be retained by the dorsal and ventral colon in the horse. Another mechanism for retention of Dy may be the "kneading" action of the gut, in which the gut mucosa engulfs particles of the 1-2 micron size in the spaces between the cells of the epithelium in the small intestine, as was suggested by Volkheimer *et al.* (1968), Volkheimer and Schulz(1968) and Sanders and Ashworthy(1961).

E. Conclusion

There were no differences between the digestibilities of dry matter, crude protein, energy and neutral-detergent fiber among the four different physical forms of alfalfa (tables II.5 and II.6). These results differ from those obtained by Haenlein *et al.* (1966) who showed differences in the digestibility of nutrients between cubes, pellets and long hay. However in his studies, the differences were only apparent when the digestibilities were based on chromic oxide. Based on total collection there were no differences. The use of Dy as a marker in the present study was inadequate. Digestibilities based on Dy were lower ($p<0.05$) than those based on the total collection method (tables II.5 and II.6). The schedule, in which intake was restricted then followed by *ad libitum* feed intake, may have caused the digestive tract to retain and release Dy at different rates during the experiment. Dy may have formed precipitates and "radiocoloids" in the environment of the digestive tract and Dy may have bound with very small feed particles or bacteria. A resultant complex of Dy may have been incorporated into the epithelium of the intestines in a manner similar to starch granules as was described by Volkheimer *et al.* (1966) or else retained in the large colon in a mannor similar to chromic oxide retention in the rumen of cows as was shown in studies by Chamberlain and Thomas (1984).

The voluntary intake trials did show a significant difference among the four physical forms. The highest voluntary intake was shown for cubed alfalfa while the other three

physical forms of alfalfa showed no significant difference among this groups.

Table II.1 Formulation of the vitamin-mineral pellets.

Ingredients	%
Ground oats	84.5
Molasses	10.8
Vitamin-mineral mixture[1]	4.7

[1]Contributed the following nutrients which supplied or exceeded the daily requirements (when 500 g of vitamin-mineral pellets were given daily: Phosphorous(monosodium phosphate), 14 g; Iron, 316 mg; Zinc, 316 mg; Copper, 71 mg; Selenium, 0.79 mg; Thiamin, 24 mg; Riboflavin, 17 mg; Panthothenic acid, 119 mg; Vitamin A, 1.2×10^4 I.U.; Vitamin D, 2.2×10^3 I.U.; Vitamin E, 119 I.U.

Table II.2 Chemical analyses of the different physical forms of afalfa on a dry matter basis.

	Long	Chopped	Cubes	Pellets
Dry matter (%)	90.8	86.9	90.7	90.0
Gross energy (MJ/kg)	16.4	16.3	16.6	16.5
Crude protein (%)	16.0	15.2	15.8	16.6
Crude fibre (%)	28.3	27.4	25.3	26.8
Acid-detergent fiber (%)	36.2	33.3	30.7	32.2
Neutral-detergent fiber (%)	40.9	38.4	38.6	39.9

Table II.3. Chemical analyses of the vitamin-mineral pellets on a dry matter basis.

Dry matter (%)	Gross energy (MJ/kg)	Crude protein (%)	Neutral detergent fiber (%)
90.7	16.46	11.9	24.8

Table II.4. Weight of the horses(kg) during the experiment.

Horse number	1	2	3	4	5	6	7	8
Dates of weighing								
May 31	547	542	556	482	541	554	475	489
June 21	549	535	523	478	524	548	465	483
July 1	540	522	542	478	518	522	542	462
August 17	550	536	550	480	527	549	468	483
Mean and standard deviation	547±3.9	534±7.3	543±12.4	480±1.7	528±8.4	543±12.5	488±31.7	479±10.3

Table II.5 Apparent Digestibilities(%) of nutrients measured by the total collection method and the voluntary intake of the different physical forms of alfalfa.

	Long	Chopped	Cubes	Pellets
Dry matter	62.8 ± 1.1[1]	61.7 ± 0.9	61.4 ± 0.8	61.7 ± 0.9
Gross energy	57.8 ± 1.3	56.9 ± 0.8	57.9 ± 0.9	57.5 ± 1.3
Crude protein	77.8 ± 0.7	77.8 ± 0.6	78.0 ± 0.8	79.1 ± 0.8
Neutral-detergent fiber	39.0 ± 0.9	37.5 ± 1.6	36.7 ± 1.7	36.4 ± 2.5
Voluntary intake kg/day	13.4 ± 0.7*a*	13.9 ± 0.8*a*	16.5 ± 0.9*b*	12.9 ± 1.0*a*

[1]Mean and standard error.

a,b Mean with the same superscripts do not differ ($P<0.05$).

Table II.6. Apparent digestibilities (%) of nutrients measured with dysprosium for the four physical forms of alfalfa.

	Long	Chopped	Cubes	Pellets
Dry matter	51.2 ± 0.9[1]	51.0 ± 2.1	51.5 ± 1.5	50.7 ± 2.2
Gross energy	44.7 ± 1.2	44.4 ± 2.0	44.4 ± 1.7	44.2 ± 2.6
Crude protein	70.9 ± 0.9	71.6 ± 1.6	72.3 ± 0.7	73.8 ± 1.2
Neutral-detergent fiber	20.8 ± 1.0	22.0 ± 3.0	21.4 ± 3.8	22.9 ± 3.0

[1]Mean and standard error.

Table II.7 Analysis of variance for voluntary intake of the four different physical forms of alfalfa.

Source of variation	Degree of freedom	Voluntary Intake		
		Means square	F	Significance of F
Animal	7	11.06	17.15	0.000[1]
Period	2	1.451	2.25	0.151
Treatment	3	8.221	12.76	0.000[1]
Error	11	0.644		

[1]Level of Significance.

Table II.8. Analysis of variance for the digestibility of dry matter, protein and energy based on total collection.

Source of variation	Degrees of freedom	Dry Matter			Crude Protein			Gross Energy		
		Mean square	F	Signif. of F	Mean square	F	Signif. of F	Mean square	F	Signif. of F
Animal	7	13.93	3.99	0.008[1]	6.79	2.17	0.087	23.29	8.28	0.000[1]
Period	3	7.33	2.10	0.136	8.09	2.59	0.084	5.48	1.95	0.158
Diet	3	1.08	0.31	0.819	7.60	2.44	0.090	1.98	0.70	0.563
Error	18	3.49			3.12			2.81		
Total	31									

[1]Level of Significance.

Table II.9. Analysis of variance of the digestibilities of neutral-detergent fiber based on total collection and dysprosium.

Source of variat.	Degrees of freedom	Total Collection			Dysprosium		
		Mean square	F	Signif. of F	Mean square	F	Signif. of F
Animal	7	58.88	4.46	0.007[1]	33.18	1.87	0.146
Period	3	19.72	1.49	0.257	407.00	22.97	0.000[1]
Diet	3	4.25	0.32	0.809	0.426	0.02	0.995
Error	15	13.21			17.72		
Total	31	37.4			21.8		

[1]Level of Significance.

Table II.10. Analysis of variance of the digestibilities of nutrients based on dysprosium.

Source of variation	Degrees of freedom	Dry Matter			Crude Protein			Gross Energy		
		Mean square	F	Signif. of F	Mean square	F	Signif. of F	Mean square	F	Signif. of F
Animal	7	14.70	1.48	0.237	8.84	1.26	0.325	18.03	1.85	0.139
Period	3	130.68	13.14	0.000[1]	35.82	5.10	0.010[1]	181.41	18.60	0.000[1]
Diet	3	11.01	0.10	0.958	12.16	1.73	0.197	0.41	0.04	0.988
Error	18	9.95			7.03			9.76		
Total	31									

[1]Level of significance.

Table II.11. Reported values and values of the present study of nutrient digestibilities(%) for alfalfa in horses and ponies.

Authors	Feed intake	Harvest condition and alfalfa form	Dry matter	Energy	Protein	Crude fibre	Organic matter
A) Horses							
Fonnesbeck	ad libitum	prebloom	58.5		59.2	36.4	
(1967)	ad libitum	1/4 bloom	52.1		65.2	39.5	
		2nd cutting		51.4			
Present Studies	maintenance	cubes, pellets, chopped and loose hay	61.9	57.5	78.2		
	maintenance	cubes	60.1	59.1	74.0		
	80% ad lib	cubes	59.3	57.0	74.0		
B)Ponies							
Haenlein et al. (1966)	ad libitum	early-bloom 3rd cutting	52		68	34	53
Slade and Hintz (1969)	maintenance	pellets alfalfa		56.9	74.0	34.7	60.4
Van der Noot et al. (1970)		1/4 bloom well fertilized	60.8	56.4	75.4		

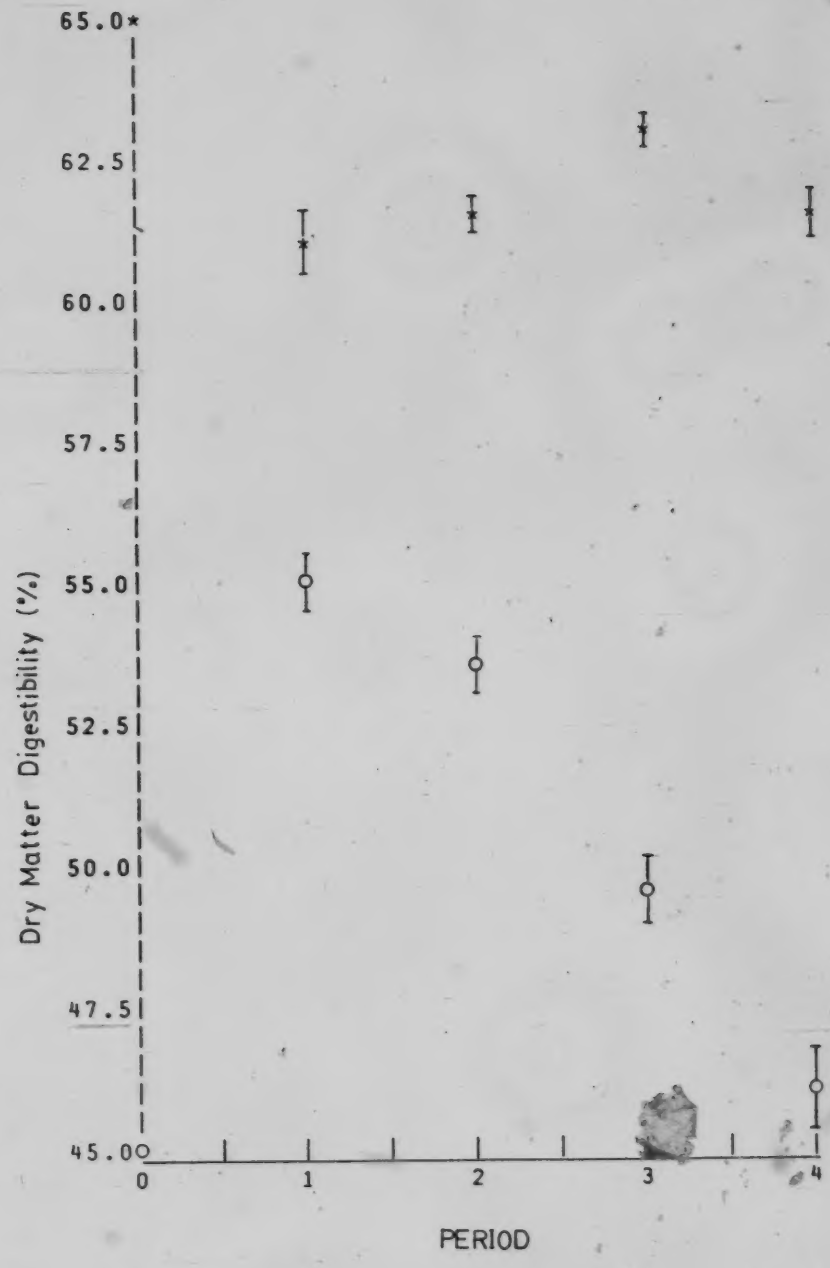

Figure II.1 The average dry matter digestibilities in each period for all four physical forms of alfalfa with standard errors as measured by dyprosium(o) and total collection(*).

Plate II.1 Metabolic cage used for total collection of feces with the urine collection funnel attached to the horse.

Plate II.2 Inside view of the metabolic cage with feces collection tub, urine funnel, harness and feeder.

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III. THE EFFECT OF LEVEL OF FEED INTAKE ON NUTRIENT DIGESTIBILITIES AND RATE OF PASSAGE IN HORSES

A. Introduction

There is a scarcity of information on the effect of the level of feed intake on nutrient digestibilities and rate of passage in horses. Nutrient digestibilities are usually determined at maintenance levels of feeding because of the improvement in accuracy of results as opposed to *ad libitum* feeding

Furthermore, studies were carried out to identify a suitable digestibility marker. Digestibility studies with horses, based on total collection, require a large amount of labour and equipment. The horses can be kept in a normal state of activity rather than being confined to a crate when digestibility markers are used. As was shown in the previous study (chapter II), the performance of a rare-earth element (dysprosium:Dy) as digestibility marker was unsatisfactory. The performance of chromic oxide was close to ideal in studies by Haenlein *et al.* (1966a,b) but less than adequate in studies by Knapka *et al.* (1967). Further studies were carried out to determine the feasibility of Cr_2O_3 as a digestibility marker.

Lastly, studies were carried out to determine the effect of level of feed intake on rate of passage. These studies were designed to relate possible changes in nutrient digestibilities to changes in rate of passage that might occur at different levels of feed intake. For this study Dy was employed as a marker for rate of passage measurements. The unsuitability of Dy as a digestibility marker does not preclude its use as a marker for estamating relative rate of passage although much work has to be carried out to establish the relatively long term retention of Dy, that had plagued the digestibility measurements in previous experiments (see chapter II), in different levels of feed intake.

B. Materials and Methods

Six mature geldings with average weight of 521 kg(standard deviation $\pm$47.5) were used to determine the effect of 2 levels of feed intake on the digestibilities of dry matter, energy and crude protein. In addition, the effect of feed intake on rate of passage was

measured. The experiment was carried out according to a randomized block design. Feed, in the form of alfalfa cubes, was supplied according to the maintenance requirement for digestible energy (D.E.)(N.R.C., 1978) as defined as D.E.(MJ/Day) = $649W^{0.75}$ and according to 80% of their *ad libitum* intake. Maintenance level of feed was calculated according to the individual weight of each horse. The *ad libitum* intake for each each horse was measured in chapter II. The horses received 500g of a vitamin-mineral mixture (in the form of pellets) each day. The horses were fed alfalfa cubes supplemented with vitamin-mineral pellets. The alfalfa cubes were commercially prepared and measured 3.3 x 3.3 x 3.3 cm.

The vitamin-mineral pellets(table III.1) provided the daily requirements of the vitamins and minerals as recommended by N.R.C.(1978). In addition, the vitamin-mineral pellets containe the two digestibility markers: Cr_2O_3 and Dy. Dy was added to the vitamin-mineral pellets to give a final dietary concentration of approximately 15 to 25 ppm. Cr_2O_3 was added to the vitamin-mineral pellets to give a final dietary concentration of approximately 0.15 to 0.25%. The vitamin-mineral pellets were fed prior to the alfalfa cubes to ensure that all of the markers that were supplied at each feeding were consumed. The horses were fed equal amounts of alfalfa cubes and vitamin-mineral pellets twice daily, at 0800 and 2000 h. Iodized salt blocks were available at all times. Chemical analyses of cubes and vitamin-mineral pellets are presented in table III.2.

The vitamin-mineral pellets (type I pellets) were prepared from a base of ground oats and 10% dry molasses (table III.1). Molasses was included in the pellets to make this part of the ration more appealing to the horse. The $DyCl_3 \cdot 6H_2O$ hydrate salt was ground very fine and sieved through a 0.5-mm mesh size screen before weighing and mixing the powder with the vitamins and minerals. Before pelleting, the vitamin-mineral marker mixture was added to a proportion of the ground oats and mixed thoroughly. Cr_2O_3 powder was then added and mixed in with the other ingredients. The resulting mixture was mixed with the remaining ground oats and molasses and pelleted. With the exception of the exclusion of Dy, the same procedure was followed in the preparation of the type II pellets.

The horses were kept in partly covered individual pens during the 14 day adaptation period. Grab samples of feces were collected at 0800 h and 2000 h during the last 3 days of the adaptation period. The horses were observed to see if coprophagy occurred. The pens were cleaned at least once a day. Thereafter, the horses were moved indoors into metabolic cages for 6 days for a total collection of urine and feces. Urine was collected separately from feces by means of a funnel-harness apparatus which was attached to the horse and which drained the urine into collection pails (plate III.1). Feces were collected by placing a large plastic container at the rear of the cage. The metabolic crates were contructed of steel and the floor was constructed from heavy wooden planks on which the horses were held 20 cm above the floor of the barn. A removable wooden feeder was placed inside the crate at the front with volume capacity for hay on the inside bottom and an upper shelf for holding the vitamin-mineral pellets and a pail of water. A block of iodized salt was placed at the bottom of the feeder. Feces were weighed at 0800 h each day during the collection period and mixed thoroughly. A subsample of approximately 500 gms was taken at random sites in the collection tub. Samples were immediately placed in a freezer at -20°C.

The type I pellets were fed during the 14 day adaptation period. The horses were fed the type II pellets from the second day onwards. Type I pellets contained the markers dysprosium and chromic oxide in quantities of 198 mg and 1.98 g, respectively, in 500 g of pellets. Type II pellets only contained the marker chromic oxide in a quantity of 1.98 g in 500 g of pellets. Serial sampling of feces was initiated at 1200 h on the second day of the collection period. Sampling was continued for 74 h (table III.3). When there was no regular defecation every 2 h then a sample was collected when it became available and the corresponding time was recorded. Only data from serial sampling from period 2 was used in rates of passage studies therefore the data was based upon three horses per feeding level.

Samples of freeze-dried feces from each of the 6 days of the collection period from each horse on each treatment (including samples obtained from serial sampling) were pooled. Samples of feed, feces and vitamin-mineral pellets were ground through a 1-mm mesh size screen using a Wiley mill and analyzed for dry matter, crude protein and energy (A.O.A.C.,

1975). Neutral-detergent fiber was determined according to Goering and van Soest (1970). The procedure for measuring the content of Dy was described under experiment 1. Cr_2O_3 was determined according to the method of Fenton and Fenton (1979). Two methods were used to determine the mean retention time: according to Faichney (1975) and Castle (1956). The mean retention time according to Faichney (1975) is calculated from equation:III.1:

$$\text{Equation III.1} \qquad \theta = T + \sum_{i=1}^{n} A_i[t_i - t_{(i-1)}]$$

Where "T" is the time elapsed between the last feeding where the marker was omitted and the defecation for which "A" becomes less than unity. "A_i" is the marker concentration in the "i"th defecation as a fraction of the equilibrium concentration and "t_i" is the time elapsed between the end of the infusion and the "i"th defecation. The method used by Castle (1956) is based on the division of the marker excretion curve into intervals of 10%, from 95% to 5% of the steady state marker concentration in feces(i.e. prior to removal of the marker). The mean retention time is then calculated by adding the ten corresponding time intervals and dividing the sum by 10.

The data for each reponse criterion were subjected to Least Squares analyses of variance (Steel and Torrie, 1980). Differences among treatment means were determined with the Student's t-test.

C. Results

All of the horses consumed the cubes when these were fed according to the digestible energy requirement for maintenance (N.R.C., 1978) calculated from the individual weights of each horse. With the exception of horse number 4 (which consumed only 55% of the estimated *ad libitum* feed intake) all horses consumed their allotted ration. The horses maintained their body weight within 6% of the initial weight during the 6 week experiment (table III.4). The horses showed no obvious health problems. The urine had a somewhat milky appearance, probably caused by the formation of calcium carbonate. The urine also contained a small amount of light brown mucus-like substances that settled on the bottom of

the pail.

There were no differences ($p>0.05$) in the digestibilities of dry matter, crude protein and energy when the cubes were fed according to the maintenance requirement for digestible energy or according to 80% *ad libitum* intake, irrespective of whether the digestibilities were based on total collection or Cr_2O_3 (table III.5). In addition, there were no differences ($p>0.05$) between mean nutrient digestibilities based on total collection and Cr_2O_3 (table III.5).

The digestibilities of dry matter were determined in grab samples collected at 0800 and 2000 h during the last 3 days of the 14 day adaptation period. The level of feed intake had no effect ($p>0.05$) on dry matter digestibilities. Dry matter digestibilities based on Cr_2O_3 were higher than based on Dy ($p<0.05$). In addition, dry matter digestibilities were lower ($p<0.05$) when determined in feces collected at 2000 than at 0800 h (table III.6).

Only the serial fecal collection from the second period was used to measure the rate of passage. Therefore horses: 3 to 5 inclusively were fed at "maintenance" level and horses: 6 to 8 were fed at "80% *ad libitum*" level. In spite of the low number of animals used in each treatment the values for the rates of passage gave a definite indication of differences between the two levels of feed intake. The marker excretion curves on which the digesta flow measurements were based upon are shown in figure III.1.

There were only small differences between mean retention times measured by both methods: 1.9 and 0.9 h when the cubes were fed at "maintenance" and at "80% *ad libitum*", respectively. There were significant differences ($p<0.05$) in mean retention times between the 2 levels of feed intake. Based on the method of Faichney (1975), the mean retention times were 24.6 and 15.3 h for the "maintenance" and "80% *ad libitum*"feeding levels respectively. Based on the method of Castle (1956), the mean retention times were 25.8 and 15.9 h, based on "maintenance" and "80% *ad libitum*" feeding respectively. Based on the begining of the decline of the marker excretion curve, the lag times were 14.2 and 8.2 h for "maintenance" and "80% *ad libitum*" feeding respectively (table III.7).

D. Discussion

Alfalfa cubes were fed at "maintenance" (approx. 7 kg/day) or at 80% *ad libitum* (approx. 11.5 kg/day). The feeding of alfalfa cubes did not appear to cause detrimental health effects. The excretion of calcium precipitates in urine that was observed in this study is a consequence of the excessive calcium intake (Caple, 1982a,b). The secretion of mucin in the urine could have been caused by a slight irritation of the urinary tract caused by the calcium crystals which may have caused the thicker mucin layer to form on the epithelium of the urethra for protection.

The level of feed intake had no effect on the digestibilities of dry matter, protein and energy. The digestibilities of nutrients were similar when based on total collection or chromic oxide (table III.5). This observation with horses is in contrast to studies on ruminants such as sheep which show a significant difference in the digestibility of dry matter when fed at different intake levels that would cause a different rate of passage (Blaxter *et al*, 1956).

The digestibility of dry matter, irrespective of if these were determined by means of Dy or Cr_2O_3 markers, were higher in grab samples obtained at 0800 than obtained at 2000 h (table III.6). These results are in contrast to those by Haenlein *et al*. (1966) who found lower dry matter digestibilities in grab samples that were collected in the morning than in the evening.

Dry matter digestibilities based on chromic oxide and determined in grab samples were in the same range to those obtained by the total collection, 56.7 verus 60.1%, respectively (tables III.5 and III.6). Dry matter digestibilities determined in grab samples collected at 0800 h were very similar to those obtained by total collection, 59.5 versus 60.1%, respectively.

As is shown in the present studies, Cr_2O_3 seems to be much more reliable as a digestibility marker than Dy. These results are in agreement with those obtained in Chapter II, in which digestibility coefficients based on Dy were lower ($p<0.01$) than those based on total collection. Dry matter digestibilities determined in grab samples were lower when these were based on Dy rather than on Cr_2O_3, 48.6 verus 56.7%, respectively (table III.6).

The possible reasons for the incomplete recovery of Dy in feces, resulting in the underestimation of nutrient digestibilities have been discussed under chapter II. There was a complete recovery of Cr_2O_3 in feces, resulting in similar nutrient digestibilities to those based on total collection (table III.5). Chromic oxide is more chemically inert than Dy which can form precipitates and "radiocolloids" with hydroxides and phosphates (Ellis, 1968; Uden *et al.*, 1980). In addition, Cr_2O_3 particles are more uniform is size, ranging from 1 to 30 microns, as opposed to Dy which can range from a few millimicrons to 1 or 2 microns (Sasson, 1966; Ellis, 1966). Furthermore, Dy can complex with organic compounds in the digesta under normal pH conditions in the digestive tract (Teeter *et al.*, 1984). Lastly, Cr_2O_3 will bind with fiber particles, in the same degree, under all the pH conditions that are found in the digestive tract. On the other hand, the binding strength of Dy and fiber particles will depend on the pH conditions in the digestive tract. Under acid conditions, the bond will be strong. Under alkaline conditions, the bond will be very weak (Hartnel and Salter, 1979).

The mean retention time when the cubes where supplied at maintenance was 24.5 h. Uden (1978) found a mean retention time of 29 h when using the liquid phase markers, CO-EDTA or Cr-EDTA. The difference in retention time between both studies may be caused by differences in diet composition, feed intake, body weight of the horses and methodology. Uden (1978) used loose timothy hay which was fed at a rate of 16.1 g per kg body weight, with two horses of an average weight of 388 kg, and determined the mean retention time with the "pulse dose" method. The horses in the present studies, with average weight of 517 kg, were fed alfalfa cubes, at a rate of 14.7 g per kg body weight.

Nutrient digestibilities were similar if these were measured at the "80% *ad libitum*" and "maintenance" level of feed intake, even though there was a large difference in rate of passage (tables III.5, III.7 and III.8).

Although there are limitations in the use of Dy as a digestibility marker, there may be merits for its use as a marker to measure rate of passage. Theureu (1970) quoted by Young *et al.* (1976) stated, "the recovery of an indicator need not be complete as long as its recovery is consistant. This allows adjustments of indicator-based estimates". The mean recoveries of

dysprosium from six horses in the second period of this study was 82.9% (standard deviation ± 9.65).

Digestibilities of nutrient measurements were similar in both the "maintenance" and "*ad libitum*" fed horses. Therefore, the proportional amount of nutrient disappearance from the digestive tract was similar in both feed levels so then the rate of fermentation in the hindgut to reduce the larger amounts of dry matter and the fiber component of the feed in the "80% *ad libitum*" diet was probably faster. The changes that occur in the large intestine of the horse to accomodate the increased fermentation rate at a higher level of feed intake may include increases in the retention of the microbial population and absorption of the intestinal secretions. An increase in the retention of the microbial population and absorption of intestinal secretion has been reported in rabbits (Bjørnhag, 1972; Bjørnhag, 1981). If the fermentation rate increases to accomodate the increase in feed intake and flow rate, then there could be a corresponding increase in the secretion of bicarbonate from the ileum and bicarbonate and phosphate from the ventral and dorsal colon to neutralize the volatile fatty acids produced. In this context, it is interesting to note that the addition of 4.5% sodium bicarbonate caused an increase ($p<0.05$) in the digestibilities of acid- and neutral-detergent fiber in studies with sheep (Mees and Merchen, 1985). An effect of the bicarbonate may be to give a more favourable environment(by buffering the V.F.A.s produced) for microbial growth and therefore increasing fiber degradation. With more secretions into the large colon, there will be a corresponding increase in water absorption in the proximal end of the dorsal and small colon. Sodium is absorbed along with water. The rate of absorption of sodium was reported to be 6 times greater at the distal rather than proximal end of the dorsal colon (Argenzio *et al.*, 1977). Along with the adjustments that occur with an increase in fermentation rate, the regulation of the rate of flow of digesta in the large intestine may be adjusted and co-ordinated, depending on the level of feed intake. The flow rate in the large intestine can be restricted at the pelvic flexture between the ventral and dorsal colon, the caeco-colic orifice and the junction between the right dorsal and the small colon (Argenzio *et al.*, 1977). Motor movements of the longitudinal band of muscles along the large intestine

co-ordinate these three areas so that they can act as sphinctors for the regulation of the flow of liquid and particulate digesta (Argenzio *et al*., 1974).

E. Conclusion

There were no differences ($p>0.05$) in the digestibilities of dry matter, crude protein and energy when alfalfa cubes were fed according to maintenance requirement as defined as D.E.(MJ/day) = $0.649W^{0.75}$ or according to 80% *ad libitum* feed intake. The mean retention time for maintenance and 80% *ad libitum* feed intake was 25 and 15 h respectively based on the method of Faichney (1975). There were no differences between nutrient digestibilities based on the total collection or Cr_2O_3 method of estamating digestibilities. Digestibility measurements of dry matter, using the Cr_2O_3 marker, on morning grab samples showed no differences from digestibility measurement of dry matter based on the total collection method. Digestibility measurements based on Dy were always consistantly and significantly lower than digestibilities based on the total collection method.

Although there are limitations in the use of Dy as a digestibility marker in horses, there may be merits for its use as a marker to measure relative rates of passage when feeding different diets at different feeding intake levels. It would be informative to use both Dy and a marker that was mordanted to fiber, in a rate of passage trial, to be able to accurately assess the absolute rate of passage of Dy relative to solid phase transit time in the digestive tract.

Table III.1 Formulation of the vitamin-mineral pellets: type I and type II.

Ingredients	%
Ground oats	79.4
Molasses	10.0
Vitamin-mineral[1] - marker mixture	10.6
Dyprosium chloride and chromic oxide (type I)	+
Chromic oxide (type II)	+

[1]Contributed the following nutrients which supplied or exceeded the daily requirements when the vitamin-mineral pellets were fed at 500g/day: Phosphorous, 5 gm; Iron, 316 mg; Zinc, 316 mg; Copper, 71 mg; Selenium 0.79 mg; Thiamin, 30 mg; Riboflavin, 22 mg; Panthothenic acid, 119 mg; Vitamin A, 1.25×10^4 I.U.; Vitamin D, 2.75×10^3 I.U.; Vitamin E, 119 I.U.

Table III.2 Chemical analyses of cubes and vitamin-mineral pellets on a dry matter basis.

	Dry matter(%)	Gross energy (MJ/kg)	Crude protein (%)	Neutral-detergent fiber (%)
Cubes	88.0	18.8	16.9	42.5
Vitamin-mineral pellets	91.0	17.7	13.1	24.8

Table III.3 Sampling Frequency: time (h) after dysprosium was omitted from the diet.

Horse number	3	4	5	6	7	8
0800 h	0	0	0	0	0	0
	4	4	5	4	4	
		6			6	6
		9	7	8	8	8
		10	10	10.5	10	11
	12		13	12.75		12.5
		14.5	14	14.5	14	14.5
	15	16	16	16	16	16
	20.75	20		20	20.2	20
	24.5	24.5	24.5	24.5	24.5	24.5
	32.8	32.2	32.2	32.2	32.2	32.2
	40		40	40	40	40
	48.2	48.2	48.2	48.2	48.2	48.2
	74	74	74	74	74	74

Serial collections for rate of passage measurements were taken during the second experimental period and horses 3 to 5 inclusively were fed at "maintenance" level while horses 6 to 8 were fed at "80% *ad libitum*" levels.

Table III.4 Weights (kg) of horses during the experiment.

Horse number	3	4	5	6	7	8
May 13	493	485	590	585	485	485
July 7	514	485	593	567	500	497

Table III.5 The digestibilities(%) of dry matter, protein and energy at two levels of feed intake and measured by total collection and chromic oxide.

	Total Collection		Chromic oxide	
Level of feed intake	80 % *ad lib*	maintenance	80 % *ad lib*	maintenance
Dry matter	57.9±2.1[1]	58.7±0.4	59.4±2.9	60.9±2.6
Protein	73.2±1.6	74.0±0.8	73.8±2.4	75.3±1.8
Energy	55.7±1.5	57.5±0.4	57.1±3.1	59.6±2.7

[1]Mean and standard error.

Table III.6. Digestibility of dry matter(%), based on Dysprosium and chromic oxide, determined in grab samples collected at 0800 and 2000 h.

Marker	Dysprosium			Chromic oxide			Overall means**
	80 % *ad lib*	maintenance	means	80 % *ad lib*	maintenance	means	
0800h	52.4±2.1[1]*a*	53.0±2.2*a*	52.7±1.4	58.5±1.6*a*	60.4±2.5*a*	59.5±1.4	56.6±1.2
2000h	42.3±2.9*b*	46.8±1.5*b*	45.3±1.8	51.8±3.9*b*	55.1±1.8*a,b*	53.4±1.9	49.0±1.5
Overall** means		48.6±1.3			56.7±1.2		

[1]Mean and standard error of the means.

a,b Mean within each marker with the same superscripts do not differ ($p>0.01$).

Table III.7. Rate of passage at two levels of feed intake.

	horse number	body weight (kg)	feed intake[1]	lag time (h)	mean retention (h)[2]	mean retention (h)[3]
maintenance	3	514	14.8	17.2	30.8	32.3
	4	485	15.5	13.4	19.6	20.6
	5	593	13.7	11.7	23.5	24.5
mean		531	14.7	14.2	24.6	25.8
80% *ad libitum*	6	567	19.4	8.0	15.3	15.0
	7	500	23.0	8.5	16.8	18.4
	8	497	20.9	8.0	13.9	14.3
mean		521	21.1	8.2	15.3	15.9

[1] g per kg body weight.
[2] according to Faichney (1975).
[3] according to Castle (1956).

Table III.8. Analysis of variance of the digestibility coefficients measured by total collection at two levels of feed intake.

Source of variation	Degrees of freedom	Dry Matter			Crude Protein			Gross Energy		
		Mean square	F	Signif. of F	Mean square	F	Signif. of F	Mean square	F	Signif. of F
Horse	5	17.11	1.11	0.458	16.70	2.304	0.190	20.93	1.38	0.367
treatment	1	1.92	1.24	0.739	1.920	0.265	0.629	9.720	0.639	0.460
Error	5	15.49			7.25			15.218		
Total	11									

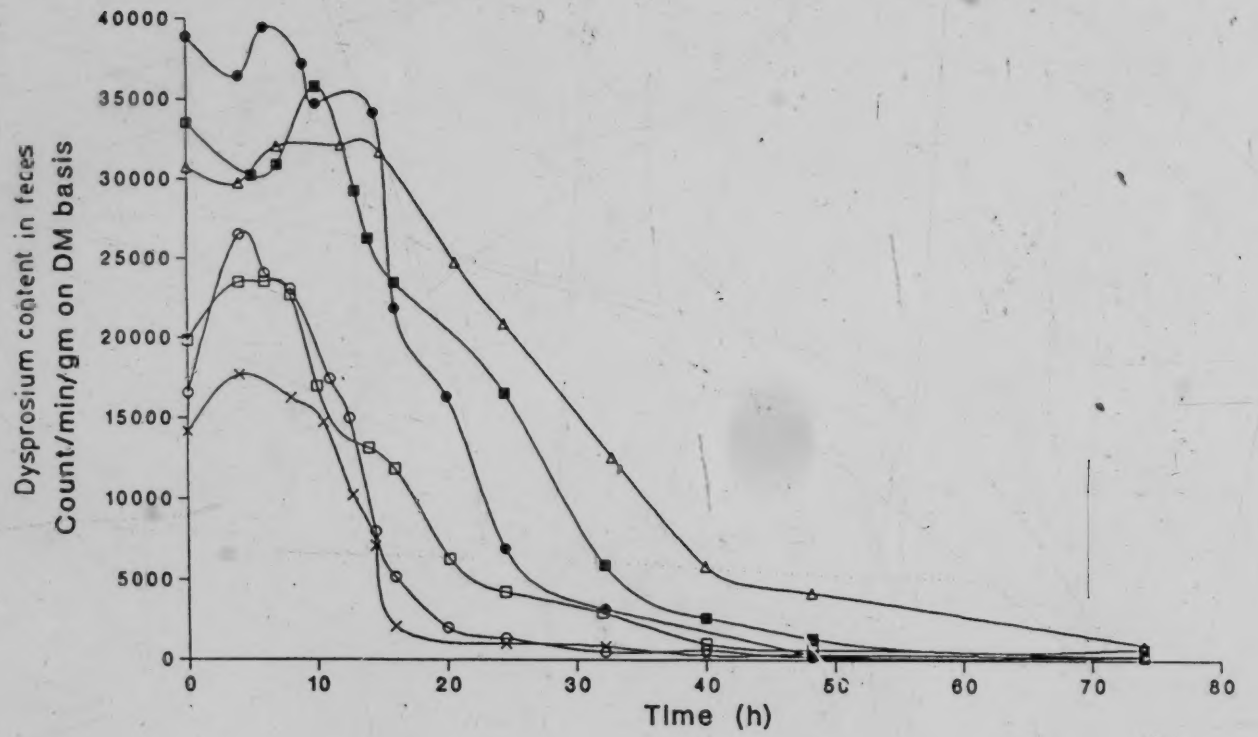

Figure II.1 Excretion curves of dysprosium in serial samples of fecal collections after removing the marker from the diet; data from the six horses in one period are used in this figure. The three curves with the highest initial value are from the horses fed at maintenance level and the three lower curves are from horses fed at 80% ad lib level.

Plate III.1 Urine collection funnel with harness for separating the urine from feces.

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IV. Discussion and Conclusions

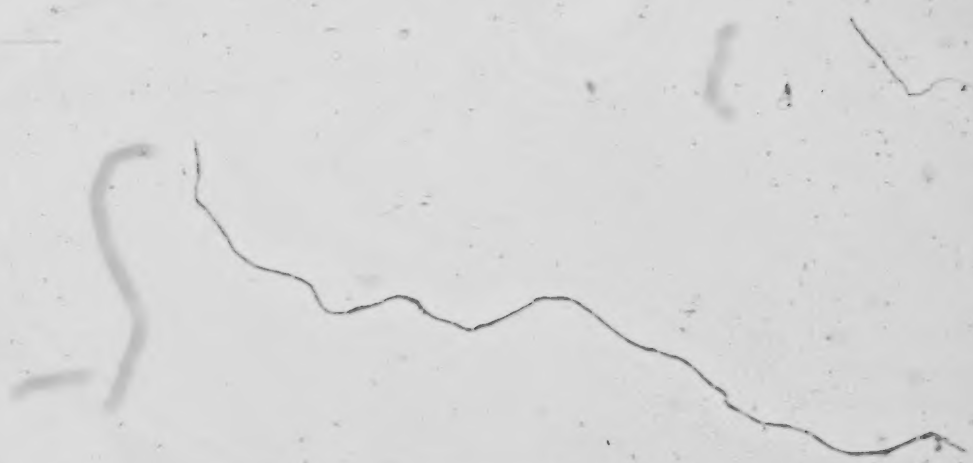
Alfalfa cubes, from two different manufactured batches, gave similar digestibilities of dry matter, protein and energy as shown in chapter II and chapter III of this present study (table II.5 and III.5). Four physical forms of alfalfa (loose, chopped, cubed and pelleted) were used in digestibility trials in chapter II. Pelleted alfalfa, which had the smallest particle size, gave digestibility measurements similar to the other three diets. It was found in the second experiment (chapter III), that digestibilities of nutrients were not affected by two levels of feed intake which gave two different rates of passage measurements (table III.7). The digestive tract of the horse seems to accommodate different size feed particles (horses have a limited ability to reduce feed particle size internally: Uden, 1978) and different feed levels (maintenance vs. 80% *ad libitum*) while still retaining a similar efficiency of digestibility (table II.5 and III.5). Chromic oxide, as an "index substance" for digestibility measurements, was found to give similar results as the total collection method in the experiment described in chapter III (table III.5).

Dysprosium chloride, as an "index substance", did not give satisfactory digestibility measurements because dysprosium recovery in the feces was low. Thus dysprosium gave lower digestibility coefficients than the total collection method in the four periods in the study of chapter II and in the two periods in the study of chapter III (table II.6 and III.6). Recovery of dysprosium during the first period of the experiment reported in chapter III was 82.9% (standard deviation $\pm$9.65) for six days of pooled total collected fecal samples using six horses.

Dysprosium seems have a predisposition for becoming sequestered in the gastrointestinal tract of the horse. Mechanisms for this may be because of "perabsorption", a phenomenon that was described by Volkheimer and Schulz (1968), in which, with the assistance of peristatsis, solid particles can become engulfed between the cells of the epithelium in the intestine.

The absolute amount of dysprosium that will flow with digesta in the digestive tract of the horse may be utilized for measuring relative rates of passage if the dysprosium retained

in the intestinal lining is predictable under various feed intake levels and for different feeds.



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